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INFLUENCE OF ENVIRONMENT AND VARIETY ON NITROGEN AND THIAMIN IN FIELD PEAS (*PISUM SATIVUM*)

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Abstract

Nitrogen and thiamin analyses of field peas (*Pisum sativum*) are presented. They include samples from the seasons 1941 to 1949 and from four States. Most of the detailed sampling comes from grain and haulm grown in the seasons 1945 to 1949 at Walpeup and Dookie, Victoria.

Environment affected nitrogen and thiamin levels through season and locality. Seed damaged by late rains in 1945 at Walpeup was much reduced in nitrogen and thiamin.

Differences in nitrogen level due to variety were highly significant. Insufficient varietal material was analysed for thiamin, but it seems likely that significant differences would result from larger sampling.

The variety Dun at Dookie showed a significant positive correlation of nitrogen and thiamin in the grain, whereas the variety Collegian at Walpeup showed no correlation. Dun also showed a slight positive correlation of thiamin and ash.

Nitrogen values of the dry haulm and pods without the seed were of the order of 1·2 to 3·8 per cent compared with wheat stubble 0·4 to 0·7 per cent.

Introduction

General

In Australia, recent emphasis on the supply of essentials for human and animal diets has stimulated fresh interest in legume crops as a source of protein and thiamin and also for their influence on soil fertility. In the period 1941 to 1943, Haughton and Coulter analysed the grain of *Pisum sativum* (field and vegetable varieties) and showed protein and thiamin levels at least twice as high as those of Australian wheats (Australian National Health and Medical Council, 1942). Further, this species is the most likely one to provide varieties of use in maintaining the soil fertility of those wheat areas of Victoria in which a pasture legume is not easily grown. Present commercial varieties yield too erratically in the drier parts of the wheat belt and so an attempt is being made to breed varieties for high yield of seed per acre. Clearly the maintenance and the increase in protein level of the crop is of importance.

In contrast to cereals, knowledge is lacking on the effect of environment and genotype on the quality of legume seed and haulm, and this is needed for better use of the present crop and for the breeding programme. Consequently data was gathered from analyses of various samples of peas grown at several localities in Victoria and elsewhere in Australia during the period 1941 to 1950. A few samples of haulm were included. This report gives some information on the factors controlling the level of protein and thiamin in the pea plant, particularly the seed.

Material

Apart from the samples obtained from commercial sources for the early analysis, material came from experimental plots concerned with varietal research on this crop. It was mostly from the Mallee Research Farm, Walpeup, and from the Agricultural College at Dookie. Walpeup has an average annual rainfall of 12·7 in.; in the years of sampling, 1945-49, it ranged from 10 in. to 15½ in. The soils are sandy. Dookie has an average rainfall of 21 in., with a range of 17-25 in. in the period 1945-49. The soils are clay loams.

The experimental plots from which the samples were selected for analyses included drilled plots of about a hundredth of an acre in size (1-2 bushel/acre in rate of seeding), and hand-sown plots, where seeds were sown about 3 in. apart in the rows. Spacing of seed along the row of the drilled plot was irregular, ranging from ½ in. to 12 in. Both drilled and hand-sown plots were sown in drills 7 in. apart, except for those varieties and crossbreds sown with wider spacing in observation rows. All sowings were made with superphosphate at a standard rate for the locality (Walpeup 90 lb./acre, Dookie 160 lb./acre). In the hand-sown plots, each plot was edged by wheat to divide each group and to reduce the border effect. All experimental plots from which samples were taken showed healthy growth. Good root nodulation was typical of the plant at both places.

Analyses

These resulted from the co-operation of a number of people. During the period 1941 to 1943, Joan M. Haughton, I. W. Robertson and J. Coulter analysed a number of varieties, and showed the existence of a wide range of values for protein and thiamin.

Over the period 1946 to 1952, the effect of variety and environment on protein and thiamin was followed in several series of samples. Through the co-operation of G. B. Jones of the Nutrition Laboratory, C.S.I.R.O., Adelaide, protein was estimated on over 200 samples of pea grain taken from the harvests of 1945 and 1946. Elizabeth Neville analysed for protein and thiamin 70 samples selected from the 1947, 1948 and 1949 harvests.

In 1952 and 1953, a number of samples, particularly of wheat and pea stubble from Walpeup, were estimated for protein by E. J. O'Brien of the Cereal Laboratory, Department of Agriculture, Victoria.

Protein was estimated from the nitrogen percentage using the factor 6·25. Other details of analytical methods are set out in Appendix I. The results of the grain analyses over the period 1941 to 1950 are summarized in Appendix II. As a matter of interest the range of these constituents in Australian wheats is added along with one set of figures on wheat in Canada. The haulm analyses are given later in the text.

The results were statistically analysed by R. Leslie, Betty Laby and Alison Doig of the Statistics Department, Melbourne University. Degree of significance is indicated by crosses (0·5, 0·1P, 0·01P being three to one cross respectively, or of high, medium and slight significance). It was recommended that further sampling on a field scale should be of the order of six to ten samples per treatment, because of the variation of result under field conditions.

Effect of Environment and Variety on Nitrogen and Thiamin of Grain

The analyses of samples from the harvests of 1945 to 1949 showed that season, locality and variety affected the nitrogen and thiamin levels.

Effect of Season at One Locality

Information on the effect of season is shown in Table 1 for Collegian at Walpeup, and in Table 2 for Dun at Dookie. At Walpeup, the effect of season is highly significant when the results for 1945 are included, and moderately significant when this year is omitted. The low value of both thiamin and nitrogen for the grain for the 1945 harvest was associated with damaged grain with a "bubbly" appearance (see later section) and low variability. There was little difference in nitrogen level in the samples from the drilled plots in the years 1946 to 1949, but the thiamin levels showed larger differences, and the lowest level in 1949 was associated with higher rainfall in September than in the other two seasons. The pea varieties are in the podding stage at this time. Table 2 shows the figures for the variety Dun at Dookie arranged according to season. In the hand-sown plots there is no significant difference in nitrogen content in spite of the range in season, and only a slight difference in the thiamin level. In the drilled plots, the nitrogen results for 1949 are distinctly lower than in 1948. The thiamin shows the same trend but is not significantly different. In 1949 there was higher rainfall in Novem-

TABLE 1

Effect of Season on Variety Collegian at Walpeup

Season	HANDSOWN					DRILLED				
	Sample No.	Nitrogen		Thiamin		Sample No.	Nitrogen		Thiamin	
		%	S.E. Mean	Mcgm/gm	S.E. Mean		%	S.E. Mean	Mcgm/gm	S.E. Mean
1945*	4	2·66	0·08	4·39	0·30					
1946	2	4·00	0·71			2	4·05	0·11		
1947						4	3·53	0·08	7·89	0·30
1948						6	3·70	0·06	8·77	0·24
1949	4	3·76	0·08	8·94	0·30	7	3·98	0·08	7·13	0·30
Significance		xxx		xxx			x		xx	

* Seed damaged by rain before harvest.

TABLE 2

Effect of Season on Variety Dun at Dookie

Season	HANDSOWN					DRILLED				
	Sample No.	Nitrogen		Thiamin		Sample No.	Nitrogen		Thiamin	
		%	S.E. Mean	mcgm gm	S.E. Mean		%	S.E. Mean	mcgm gm	S.E. Mean
1945	3	3·62	0·09	7·69	0·34					
1946	4	3·96	0·08							
1947	2	3·62	0·11	9·36	0·42					
1948	3	3·79	0·09	9·84	0·34	8	3·58	0·06	8·91	0·21
1949	6	3·66	0·06	9·75	0·24	3	2·82	0·09	8·11	0·34
Significance		N.S.		x			xxx			

TABLE 3

Effect of Locality on Nitrogen and Thiamin in Grain of Pea Varieties

Year	Variety	WALPEUP					DOOKIE					Type Plot
		Sample No.	Nitrogen		Thiamin		Sample No.	Nitrogen		Thiamin		
			%	S.E. Mean	mcgm/gm	S.E. Mean		%	S.E. Mean	mcgm/gm	S.E. Mean	
1945	Collegian ..	10	2·63	0·05			3	3·79***	0·09			Handsown Plot
	White Brunswick	6	2·82	0·06			6	3·92***	0·06			,, ,,
	M.U. 33 ..	7	3·06	0·06			4	3·76***	0·08			,, ,,
1946	Collegian ..	2	4·00	0·11			4	4·06	0·08			,, ,,
	W. Brunswick ..	4	4·06	0·08			3	3·97	0·09			,, ,,
	,, ,, ..	4	4·58**	0·08			4	4·00	0·08			Handsown Row
	M.U. 225 ..	2	4·23**	0·11			5	3·75	0·07			Handsown Plot
	224 C ..	2	4·41**	0·11			5	3·75	0·07			,, ,,
	224 A ..	2	4·46**	0·11			4	3·74	0·08			,, ,,
	231 ..	4	4·21**	0·08			4	3·62	0·08			,, ,,
1947	Collegian ..	4	3·53	0·08	7·89	0·30	1	3·49		8·44		Drilled
1949	W. Brunswick ..	1	3·69		9·73		2	4·28	0·11	12·85	0·42	Handsown Plots
	Collegian ..	4	3·76	0·08	8·94	0·30	2	3·80	0·11	11·35**	0·42	,, ,,
	M.U. 33 ..	1	3·60		7·89		1	3·87		9·75		,, ,,
	Mammoth Blue	1	4·11		12·40		2	3·93	0·11	12·85	0·42	,, ,,
*Wheats—4 Varieties** ..												
1941	Bencubbin ..		2·6		5·6			1·5		4·6		
	Dundee ..		2·5		6·6			1·6		5·0		Drilled Plots
	Ranee		2·2		5·5			1·6		4·6		
	Ford		2·6		5·5			1·9		5·6		
1942	Bencubbin ..		2·1		5·2			1·3		4·9		
	Dundee ..		2·0		5·2			1·4		6·3		
	Ranee		1·8		5·2			1·5		4·2		
	Ford		2·0		5·3			1·5		5·8		

* Aust. National Health and Medical Research Council (1942).

ber than in other years and it included fairly heavy rain over three days towards the end of the month. It is likely that some damage to seed occurred in the rain-flattened drilled plots, while the small hand-sown plots were held erect by their boundaries of wheat.

In addition to the effect of season, small significant differences were found between nitrogen and thiamin levels of the same variety sown in hand-sown plots compared with drilled. These differences might result from fertilizer placement and suggest an investigation of the effect of nutrient supply on nitrogen and thiamin levels of the grain. Several workers (Langston 1951, Burkholder and McVeigh 1940, and Finch and Underwood 1951) have shown that such constituents of a cereal seed can be affected by the supply of nitrogen, phosphorus, sulphur and other elements to the root.

Effect of Locality

In Table 3, data is given for the same varieties grown at both Walpeup and Dookie in the same years. Figures for four wheat varieties are added for comparison (Australian National Medical and Research Council, 1942). It must be remembered that peas grown at Walpeup were manured at half the phosphate rate of those at Dookie.

Because of the shorter growing season at Walpeup the pea varieties grown there were predominantly of early maturity. Varieties of the mid-season type such as Dun, suited to the conditions at Dookie, only yield well at Walpeup in the exceptionally late season, and so rarely produced seed needed for these analyses. The New Zealand variety, Mammoth Blue, is the only one of such a type in Table 3. The number of samples of each variety is too low in some cases for statistical treatment, but general trends can be seen. The nitrogen figures show that results vary according to year. In 1945, three varieties are significantly higher at Dookie than at Walpeup because of the seed damage at the latter place. In 1946 five out of six varieties were higher at Walpeup than at Dookie. This same effect is to be noted in the wheat varieties for the two years quoted and has been found consistent for other years. However, for peas, further testing is needed to show if a similar increase of protein is usual at Walpeup compared with Dookie. Considering the thiamin figures, Collegian in 1949 was significantly higher at Dookie than at Walpeup. The three other varieties listed show the same trend.

Effect of Variety

Significant differences of nitrogen level due to variety were shown for some varieties and crossbreds in the seasons 1945 and 1946 at both Dookie and Walpeup. The results are listed in Table 4. As in wheat, the differences were often of the

TABLE 4

Effect of Variety on Nitrogen of Grain

Year	Place	Plot Type	Variety No.	Nitrogen Percentage Range of Means	Significance
1945	Walpeup	Handsown	6	2·6 — 3·1	xx
1945	Dookie	,,	6	3·5 — 3·9	xx
1945	,,	Broadcast	3	3·6 — 4·0	xxx
1946	Walpeup	Handsown	8	4·0 — 4·6	N.S.
1946	Dookie	,,	7	3·7 — 4·0	xx

same order as those due to season and to locality in the one variety. Varietal differences in thiamin level have not been adequately tested as yet, the only figures being those listed in Table 3.

Association of Thiamin with Nitrogen and with Ash

Interest was aroused in the possible association of thiamin with nitrogen and with ash in the pea grain through the report of such associations in cereals, especially wheat. Sufficient pea analyses were available only for Dun at Dookie and Collegian at Walpeup.

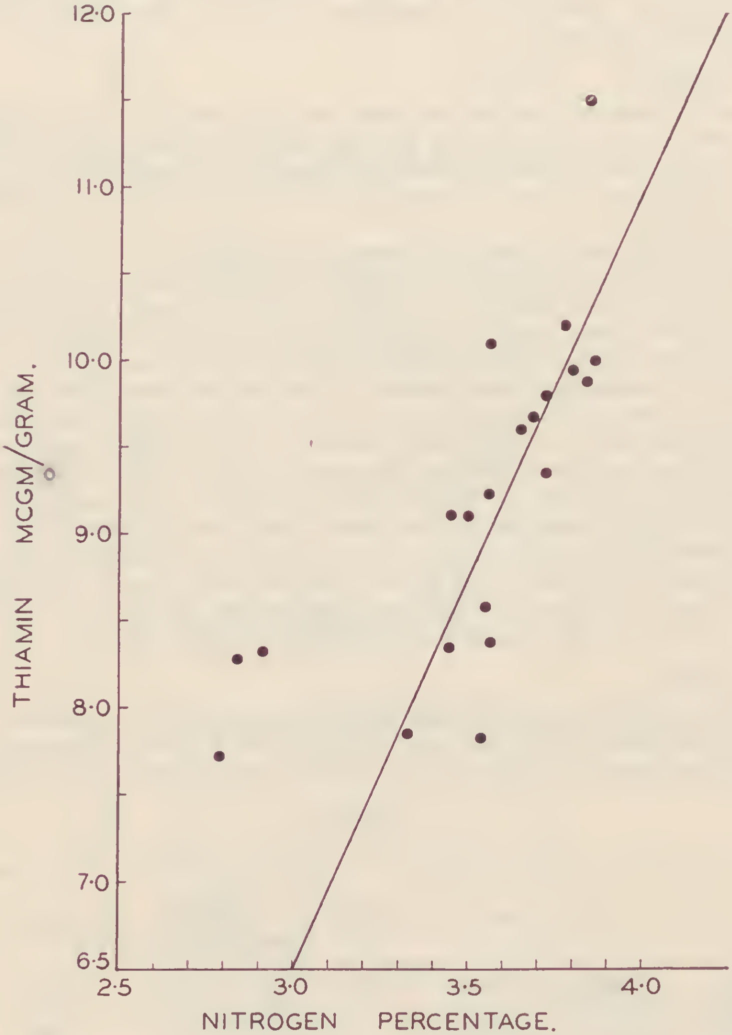

FIG. 1.—Nitrogen and thiamin in pea grain, variety Dun, at Dookie. Correlation, $r = +0{\cdot}79$.

Thiamin and Nitrogen

A highly significant correlation ($\tau = +0{\cdot}73$) of thiamin with nitrogen was found for the variety Dun at Dookie for the years 1947 to 1949, but only a low correlation ($\tau = +0{\cdot}33$) for Collegian at Walpeup. Figure 1 shows the scatter diagram for Dun at Dookie. The three values in the bottom left-hand corner are from the 1949 drilled plots, and therefore from samples that included damaged seed. If these values are left out, the correlation is higher still ($\tau = +0.79$).

Thiamin and Ash

The values for Dun at Dookie showed a correlation of $\tau = +0{\cdot}42$ (0·01P), while those for Collegian at Walpeup had a correlation of $\tau = +0{\cdot}20$ (not significant).

Distribution of Nitrogen and Thiamin in Grain and in Haulm

For certain uses of the pea crop, knowledge is needed not only of the value of the whole grain, but also of the distribution in the grain of nitrogen and thiamin. In addition, the nitrogen and fibre in the rest of the plant needs study if its value for grazing purposes is to be assessed. Some information on these points has resulted from local research in 1941-42, and from recent analyses.

Normal Grain

Figures concerning the distribution of nitrogen and thiamin within the grain are given in Table 5. Joan M. Haughton (1942) separated the seedcoat from the cotyledons and embryos of samples of two commercial varieties of field pea, Dun and White Brunswick. The seedcoats contained little nitrogen. However, samples of pea pollard and split peas produced commercially by crushing bulk supplies proved both to be similar in nitrogen level to the whole grain. The pea pollard is made up of seedcoat and of the outer edges of the cotyledons rubbed off in the

TABLE 5

Distribution of Nitrogen and Thiamin in Grain of Pea and Wheat

Analyst	Variety	Sample Type	Nitrogen %	Thiamin mcgm/gm
	Peas			
Haughton	Dun	Cotyledon	4·0	8·5, 11·1
		Testa	0·6	—
		Pollard	4·8	7·5
		Split Peas	4·5	10·3
Coulter	Tasmanian Blue	Embryo		11·2
		Cotyledon		7·9
		Outside layer of cotyledon		8·8
		Testa		0
	Wheat			
O'Brien + ...		Pollard	2·1	13·2
Clare & Underwood*		Grain	1·5	3·7

+ O'Brien: Nitrogen analyses F.A.Q. Victorian Wheat, mean 8 years, 1944/45 to 1953/54.
* Clare & Underwood: Thiamin analyses W.A. wheat from 4 mills 1947.

crushing process. As the seedcoats are so low in nitrogen, it is presumed that the outside layers of the pea cotyledon are somewhat higher in nitrogen than the inner layers, to result in the level of the pea pollard being equivalent to that of the split peas. Contrasting with wheat, there is no localization of protein granules in the outer layers of the cotyledon and therefore no great superiority of nitrogen level in the pollard compared with the rest of the grain.

Thiamin estimates done by J. Coulter (1943) showed that for the varieties Dun and Tasmanian Blue there was a higher level of thiamin in the tissue of the embryo than in that of the cotyledons. Scrapings of the outside layers of the cotyledons showed a slightly higher level there also. The testa had none. Commercial samples of pea pollard and split peas derived from Dun variety showed a lower value of thiamin in the Pollard compared with that in the split peas. Wheat pollard, on the other hand, was much higher than the whole grain. This points to a more even distribution of thiamin in the pea grain than in the wheat.

"Bubbly" Grain

The damaged grain from the 1945 harvest at Walpeup showed localized swellings of the seedcoat and, below these, circular white areas extending into the tissue of the cotyledon. Microscopically, the white areas differed from the normal tissue in the separation of many of the cells from each other and in the presence of free starch grains. Appropriate staining showed no sign of bacterial cells or fungal hyphae. The floury white material from these areas was extracted by means of a drill in sufficient quantity for estimation of nitrogen. This was compared with that from non-damaged areas. One sample was taken from typically damaged seeds of one variety from the hand-sown plots at Walpeup, all of the varieties tested being affected badly. The other samples were from a single plant from the F2 crossbreds at Dookie, where such damage was occasional. Table 6 shows that the

TABLE 6

Nitrogen Content of Grain, Season 1945

Locality	Sample Type	Nitrogen %
Dookie	"Bubbly" seed	3·2
	Normal	4·0
Walpeup	Floury patches in cotyledon of "bubbly" seed	2·0
	Normal sections of cotyledon of "bubbly" seed	3·9

nitrogen percentage was distinctly lower in the damaged tissue of the damaged seed. In Table 1 it can be seen that the thiamin content of damaged seed at Walpeup was well below that of the range for the other seasons, and the order of reduction was even more than that for the nitrogen. It seems reasonable to conclude that the reduction was from the same cause. Examination of the weather records for that season showed that the only unusual feature was several consecutive days of rain towards the end of the maturing stage of the crop. Table 7 shows the occurrences of a prolonged rainy spell late in October and those of damaged ("bubbly") seed over the seasons 1939 to 1952. Pea plots sown from May to June are ready for harvesting by the first week in November, or slightly before this. Rain during October may be too late to benefit the crop, and even makes possible fungal attack on the dying or dead tissue of leaf and stem and pod,

TABLE 7

Relation of Rainy Weather (October) to Bubbly Seed, Walpeup, 1945 to 1952

Season	Incidence Rain on Three or More Consecutive Days			Pea Grain
	No. days	Date	Amount (Pts)	
1945	5	26–30/10	130	Most seeds bubbly
1947	5, 3	1–5/10; 20–22/10	60; 70	Normal
1948	4	11–14/10	160	Normal
1949	5, 5, 4	2–6/10; 12–16/10; 23–26/10	80, 56, 90	Some bubbly seed
1951	4	22–25/10	40	Normal
1952	3	25–27/10	80	Some bubbly seed

and damage to the maturing or mature seed inside the pod. The damage to the seed described above as "bubbly" has not been produced experimentally so far, so that it is not yet known whether wetting and drying the mature pod several times before the seeds are dry, or just afterwards, is the necessary condition for the damage. The appearance is similar to "mottled" grain in wheat, a condition greatly influenced by environment, particularly by the rate of drying-out of the grain before harvest (Raw, 1932).

Seed damaged to the drastic extent of that from the 1945 harvest has not occurred again in the period 1939 to 1952, therefore this damage is regarded as

TABLE 8

Nitrogen and Fibre Percentages of Pea and Wheat Haulm

Analyst	Variety	Place	Type	Sample No.	Nitrogen		Fibre	
					Mean	Range	Mean	Range
	Pea Haulm							
Neville		Dookie 1949	Dry Mature	3	2·5	2·4–2·6	38·3	36·6–39·5
,,	Crossbred ..	Walpeup 1949	,,	4	3·1	1·9–3·8	24·0	21·0–32·9
O'Brien	Collegian ..	Walpeup 1951	,,	4	1·3	1·2–1·6		
,,	Collegian ..	Walpeup 1952	,,	5	1·3	1·1–1·7		
,,	McKay ..	Dookie 1952	Green Mature	1	1·7			
Morrison	Unknown ..	America	Hay	35	2·4		17	
			Straw	23	1·0		33	
	Wheat Haulm							
O'Brien	Insignia ..	Walpeup 1951	Dry Mature	8	0·5	0·4–0·7		
,,	Insignia ..	Walpeup 1952	,,	4	0·5	0·4–0·6		

"Haulm" includes leaf and stem but excludes pea pods and seed, or cereal ear.

an occasional hazard to the pea crop in the Mallee. Wheat grain from the 1945 Walpeup harvest was normal in appearance and, as usual, higher in nitrogen than that from Dookie. It was clear that the seasonal conditions were damaging for the pea crop only.

Haulm

The value of the pea plant apart from its grain is considered in terms of nitrogen only, because of the difficulty encountered in estimation of thiamin. The thiamin content is likely to be relevant to the nutrition of non-ruminants but of less value to ruminant species (Blaxter 1954). Table 8 shows nitrogen and fibre determinations on the dry pea plant, excluding the seed, done by Elizabeth Neville and by the Cereal Laboratory of the Department of Agriculture, Victoria. An overseas estimation is added (Morrison 1950) for comparison.

Those from Walpeup wheat and pea plots in 1951 and 1952 can be compared with samples of wheat stubble taken from the same test. The nitrogen level of the pea haulm is nearly three times that of the wheat. The 1952 samples of pea haulm included several with much fungal attack on leaf and stem. The nitrogen level was reduced slightly, but was of the same order as that found in the generally undamaged samples of the previous season.

The sample from Dookie taken from a variety of long growing season, and at the stage just before drying, is of the same level of protein as the best sample of Collegian at Walpeup.

Discussion

The preceding information shows that—

(a) pea grain and haulm are higher in nitrogen and thiamin than wheat grain and stubble, likewise pea grain is higher in thiamin than wheat grain;

(b) environment affects the level of nitrogen and thiamin in pea grain, and of nitrogen in pea haulm;

(d) thiamin is associated with nitrogen and with ash in the variety Dun.

(a) Several lines of research suggest that the high level of nitrogen and thiamin typical of the pea grain is due to a high synthetic capacity and to effective nodulation. Mulder (1948) reported that un-nodulated pea plants died early at low levels of nitrogen supply whereas oat plants produced seed, and at higher levels of nitrogen peas developed a concentration of nitrogen almost 25% higher than that in oats. Bonner and Green (1939) found that peas and tomatoes had a higher thiamin concentration in their leaves than some grasses. McCrae (1943) found that the thiamin level of the green parts of peas at flowering was 5 micrograms per gram, while Geddes and Levine (1942) recorded 3 micrograms per gram in the tiller of wheat at the same stage.

The importance of effective nodulation to protein level has been shown for the soybean by Hampton and Albrecht (1944) and for the peanut by Thornton and Broadbent (1948). In the peanut, the nitrogen of the foliage of nodulated plants was 1.8% compared with 0·9% in un-nodulated ones. That of the fruit was 4·0% and 2·6% respectively. As yet, the relation of nodulation to thiamin concentration has not been reported but it is known that nitrogen supply can affect thiamin concentration in wheat (Greer and Kent 1950) and oats (Hunt *et al.* 1952).

(b) *Grain.* Season affected strongly the nitrogen and thiamin of the grain when seed tissue was damaged by late rains, as in 1945 at Walpeup. However in the years 1946 to 1949 the only likely relationship between season and these constituents was that of low thiamin and moist spring weather. In cereals, Paull and Anderson (1942) and O'Donnell and Bayfield (1947) have shown an association of weather at flowering time with both protein and thiamin contents of the seed, moist spring weather being connected with high thiamin.

Locality affected both nitrogen and thiamin level. The higher level of nitrogen in the pea at Walpeup in 1946 compared with that at Dookie is typical of wheat grain from these two places. It is likely to be due to the shorter period for seed development at Walpeup. In 1945 the pea grain at Walpeup was lower in nitrogen and thiamin than that of Dookie because of the seed damage in that season. The possibility of such gross damage by late rains suggests the need for rapid harvesting of the pea crop after maturation. The rarity of such damage points to the acceptance of such a risk if the pea crop is more valuable to the farmer as dry grazing material in the paddock than for harvested grain. In 1949, thiamin was higher at Dookie than at Walpeup. Information is needed from other years before it can be seen if this is usual. "High thiamin" areas have been found for wheat in Canada (Johannsen and Rich 1942, McElroy *et al.* 1948), in the United States (Robinson *et al.* 1949) and in England (Greer *et al.* 1952), and it is possible that they might occur for other plant species.

Haulm. The grazing value of the pea haulm depends mainly on its nitrogen and fibre contents at maturation and then on the damage to the tissue by disease or by mechanical means, either of which can reduce the nitrogen in relation to the amount of fibre. In 1949 the higher nitrogen and lower fibre content at Walpeup compared with Dookie is likely to have been the result of careful plant sampling at the former compared with sampling from a raked drilled plot at the latter place. The low estimate by Morrison (1950) of nitrogen in pea straw could be due to mechanical loss of leaf before sampling.

In 1951, four samples of Collegian at Walpeup were damaged by fungal attack on the mature plants following late rains, and one was almost free of it. The latter had the highest nitrogen value but the others were of the same order as the healthy samples from the previous seasons. This suggests that a moderate amount of fungal damage makes little difference to the grazing value of the haulm.

There is overseas evidence of increased level in the haulm of Australian winter peas and vetches with calcium and phosphate fertilizers (Davis and Brewer 1940).

Concerning the effect of variety on the value of pea haulm, it may be concluded provisionally to be of no importance, from the similarity in nitrogen level of the haulm of Mackay, a late flowering variety, and that of Collegian, an early flowering variety. On the other hand, varietal differences in protein level have been found for lucerne (Stuker and Crandall 1953), and the possibility of such should not be ignored for peas.

Evidence that fertilizers can affect the nitrogen content of pea grain (Vidalon *et al.* 1948, nitrogen and calcium fertilizers) and of pea haulm (Davis and Brewer 1940, calcium and phosphate fertilizers) suggests the investigation of nutrient supply in relation to nitrogen and thiamin levels of the field pea grain and haulm.

(c) The occurrence of genetical variation in nitrogen content of the pea grain in the small number of varieties sampled suggests that further testing should be done on other varieties. Weiss, Weber and Williams (1952) found a range of 6% to 7% nitrogen in soybean seeds according to variety. Thiamin estimates should be included on further analyses of pea varieties, as genetical variation in this character has been found for two varieties of peas (Murray 1948) and for such widely different species as peanuts (Reddi 1949) and wheat (O'Donnell and Bayfield 1941).

(d) The significant correlations of thiamin with nitrogen and ash for the variety Dun at Dookie are the first evidence of such associations in a legume seed. It is interesting to note that Lee and Underwood (1950) found significant positive correlations between nitrogen and thiamin for each of two wheat varieties grown in several districts and seasons in Western Australia. However, research in Canada (Whiteside and Jackson 1943, Spencer and Galgan 1949) showed that this association was affected by variety, as it appears to be with peas. Variation in the correlation of thiamin and ash according to variety of wheat was also found by Robinson and others (1949).

Explanation of both the existence of correlations and of the effect of variety must wait on adequate physiological knowledge of the relationships between these constituents.

From the above discussion it can be concluded that the factors controlling the levels of protein and of thiamin in the pea plant are partly genetic and partly environmental. The high level compared with wheat is likely to be due to a superior capacity for synthesis of both these constituents and to effective nodulation supplying nitrogen for the higher amounts possible in this species.

Detailed investigations on the effect of nodulation, nutrients, season and locality are needed to enable a thorough understanding of the factors affecting protein and thiamin in the pea crop, and so the most efficient maintenance of high levels in these characters.

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Appendix I

Analytical Methods

Estimates on Grain

Each sample of grain was not less than 20 grams (80-100 seeds), to allow for determinations. Each was ground in a Christie and Norris laboratory mill (perforated sheet 1/32 in. diameter, 135/sq. in.). In the resulting flour the epidermis was still visible as small flakes (1 mm. square); the material was thought to be sufficiently finely divided for extraction of soluble materials. Samples were ground when received and stored in airtight containers at room temperature. No deterioration was detected in check samples assayed immediately after grinding and again when the last determinations had been made (up to six months). Eight samples from the Nutrition Laboratory's series, done towards the end of 1946, were checked by Miss Neville in 1950-51 with no difference in result.

Analyses are given on oven dry basis. Moisture was determined by drying at 104° C. to constant weight, $\pm 0{\cdot}5\%$. Nitrogen was estimated by the micro-Kjeldahl method with an error of $\pm 1\%$. The factor of 6·25 was used to give crude protein percentage.

Thiamin was estimated by a chemical method, a modification of the thiochrome method of Comner and Straub (1941). Removal of starch and liberation of thiamin from co-carboxylase was carried out by incubating with takadiastase at pH 4·5. Fluorescence in the final solution was read in a Beckman fluorimeter, using a Wratten 49A filter between the solution and photocell. Extraction with 0·06 N HCl at 100° C. was found to be necessary before the enzymatic hydrolysis. The extract showed an intense white fluorescence under ultra-violet irradiation; washing with isobutanol removed material having a blue fluorescence in isobutanol, so that the washed extract had only a small fluorescence within the wavelength range to which the photocell responded. This procedure gave a low blank for unoxidised extracts. However, estimation of the percentage of the true thiamin content obtained by the above procedure (by adding standard thiamin solutions at various concentration levels to the pea-meal at the beginning of the extraction process) suggested that only 85-90% was being recovered. Experiment showed that the fraction lost was adsorbed on the surface of the pea residue remaining after incubation, and that 100% recoveries could be achieved by adding concentrated HCl after incubation to lower the pH to approximately 1 before separation of the extract from the residue. Figures given are averages of three determinations, the standard deviation for the estimation being 1·5/100 units.

Estimations on Haulm

The air-dried material was ground to a fine chaff in the same mill. Nitrogen was estimated as for grain. The thiochrome method was found unsuitable for assaying thiamin in this material; no reliable method could be found for removing interfering substances. Fibre was determined by the A.O.A.C. method.

Appendix II

NITROGEN AND THIAMIN IN PEA GRAIN COMPARED WITH WHEAT

Reference	Material Source and Type	Laboratory	No. of Samples	Nitrogen %		Thiamin %	
				Range	Mean	Range	Mean
Pea Material:							
Haughton ..	Vic., Tas., 1941 Plots and Crops	Melbourne Biochemistry School	8	3·8–5·0	4·2	6·2–13·7	10·2
Coulter ..	Vic., N.S.W., Tas., S.A., 1941 Crops	,,	45			6·4–12·8	8·2
Jones	Vic., Plots 1945	Nutrition Laboratory Adelaide	114	2·7–4·4	3·4		
Jones	Vic., Plots 1946	,,	111	3·4–5·1	4 0		
Neville ..	Vic., 1945 Plots	Melbourne Biochemistry School	7	2·6–3·7	3·0	5·4–8·0	6·1
Neville ..	Vic., 1947–9 ..	,,	60	2·8–4·9	3·7	6·3–13·6	7·2
Snook	W.A. Crops White Brunswick	Depart. Agriculture W.A.	8	3·6–4·1	3·8		
Wheat Material:							
Bottomley ..	Vic. Crop silo delivery samples 1936/7 to 1940/41	Kimpton's Mill Melbourne	250	1·3–2·2			
Aust. Nat. Health & Med. Res.	Aust., Plots 1940/41	Anatomy School Canberra	260+	1·2–2·6	2·0	3·8–6·9	5·3
,,	Plots 1941/42 ..	Biochemistry School Melbourne	45	1·4–2·2	1·7	3·5–6·3	5·0
Finch & Underwood	Vic., W.A., Plots 1949	Agriculture School Perth	49	1·4–2·3	1·8	2·5–5·2	3·9
,,	Dookie, Quadrat 1949	Agriculture School Perth	8	1·7–2·3	2·0	3·4–4·6	3·9
Lee and Underwood	W.A., Plots, 3 Districts, 1949	Agriculture School Perth	50+	1·6–2·1	1·9	3·2–4·1	5·1
Whiteside & Jackson	Western Canada Hard Red Spring Wheats 1940	Whiteside & Jackson	160+	2·2–2·6	2·4	4·0–7·1	5·0